

# CYANIDE TOLERANCE IN MILLIPEDES: COMPARISON OF RESPIRATION IN MILLIPEDES AND INSECTS<sup>1</sup>

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Several years ago, specimens of the millipede *Pleurolooma flavipes butleri* (McNeill) were brought to the Department of Entomology at Purdue. Upon confinement in cyanide killing jars, the millipedes remained alive and active. When handled they produced a volatile compound with the almond-like smell of cyanide. After placing a millipede in a small closed test-tube with several german cockroaches (*Blattella germanica* (L.)), the insects died within a short time while the millipede remained unharmed. Further investigation showed that *P. f. butleri* was considerably more tolerant than *B. germanica* to injected potassium cyanide (Hall, 1967). These observations suggested that like some other millipedes (Eisner and Meinwald, 1966) *P. f. butleri* produced hydrogen cyanide as a defensive compound and raised the intriguing question of how these animals survive their own toxic secretions.

The degree of resistance to cyanide in an organism has frequently been correlated with its level of respiration. Thus Bodine and Boell (1938) report that sensitivity of diapausing *Melanoplus* eggs to cyanide increases with their rate of respiration and very low respiratory activity has been reported for cyanide-insensitive diapausing lepidopterous pupae (Schneiderman and Williams, 1954). A biochemical basis for the dependence of cyanide sensitivity on rate of oxygen consumption is provided by the excess terminal oxidase hypothesis (Kurland and Schneiderman, 1959). On the other hand Yust and Sheldon (1952) report that in scale insects which were highly tolerant to cyanide, the male pupae had a high respiration rate which did not differ from that of susceptible pupae. There is little data on millipede respiration but reports suggest that they too may have low rates. This paper is therefore concerned with investigation of the nature of the toxic defensive secretion of *P. f. butleri* and a second, cyanide-producing millipede *Euryurus leachi* (Gray) and with assessing the respira-

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tion rates of these millipedes in contrast to two cockroaches, *B. germanica* and *Blaberus discoidalis* (Serville).

A more detailed report of the biochemical basis for the tolerance mechanism will be published elsewhere.

#### EXPERIMENTAL ANIMALS

Specimens of *E. leachii* were collected from rotting logs in summer and fall of 1965 and the spring of 1966 at McCormick's Creek State Park, Spencer, Indiana. Specimens of *P. f. butleri* were obtained from large migratory populations in the summer and fall of 1965 in Lakeville and Martinsville, Indiana. Females outnumbered males by about 10 to 1. A third migratory population, largely of last instar larvae, was sampled in Columbus, Indiana in summer, 1966.

Both species were reared in covered clear plastic boxes containing 2 to 3 inches of decaying wood and leaf litter which was kept moist by periodical addition of water. *E. leachii* prospered and a year round colony was maintained but it was not possible to maintain the colony of *P. f. butleri* for more than a few months under these conditions.

*B. germanica* and *B. discoidalis* were laboratory strains reared on Gaines dog food and water. Both the insects and millipedes were maintained at 65–75° F with a 12 hour light-dark cycle.

#### IDENTIFICATION OF HYDROGEN CYANIDE AS A SECRETORY PRODUCT OF THE MILLIPEDES

Adults and immatures of both *P. f. butleri* and *E. leachii* when disturbed emit a colorless liquid with a pungent almond-like odor from small openings on the dorsal surface near the tips of some of the notal projections. Such secretory ducts are located in both species on segments 5, 7, 9, 10, 12, 13, 15, 16, 17, 18 and 19 of adult males and females. A series of semimicro qualitative spot tests for hydrogen cyanide (Feigl, 1958) were carried out on these secretions. The tests were replicated and appropriate blanks and standard solutions were included.

*Picric Acid Test.*—The defensive secretion was collected directly on small pieces of filter paper treated with a saturated solution of picric acid and sodium carbonate solution. A red color developed which indicated the presence of cyanide.

*Starch-Iodine Test.*—The test is based on the combination of cyanide and iodine to form colorless cyanogen iodide. Ten adults of each species were placed in stoppered 50 ml flasks. After shaking the flasks vigorously

for a few seconds, a blue potassium iodide-starch paper treated with 0.1 N iodine was inserted. Rapid discharge of the color again indicated cyanide in the atmosphere of the flask.

*Palladium-Dimethylglyoxime Test.*—A drop of the secretion was mixed with alkaline palladium dimethylglyoxime and nickel ammonium chloride. The formation of a red complex indicated the presence of cyanide.

This combination of positive tests strongly indicates the presence of cyanide in the defensive secretions of both *P. f. butleri* and *E. leachii*. It has been suggested that species of *Pleurolooma* release HCN under natural conditions (Young (1958)) but the presence of cyanide in the secretions of *E. leachii* has not been previously reported. Probably the repugnatorial gland secretions also contained compounds other than HCN (Eisner and Meinwald, 1966) but these were not examined.

#### RESPIRATION RATES

Respiration was determined in terms of oxygen consumption by Warburg respirometry, using standard manometric techniques (Umbreit, 1964). Individuals of *P. f. butleri*, *E. leachii* or *B. germanica* were placed in 20 ml Warburg flasks with a center-well containing a filter paper wick dipping in 0.2 ml of 20 per cent KOH. *B. discoidalis* were placed in special Warburg flasks with a final volume of 100 ml and containing 0.5 ml 20 per cent KOH. In each case, correction was made for the volume of the animals in the flask. Both male and female *E. leachii* were found to produce an unknown volatile compound, not absorbed by the KOH trap, which led to erroneous results. The release of this material was prevented by waxing over the secretory ducts with a mixture of 1 part beeswax and 1 part petroleum jelly. The millipedes were left about 30 minutes after this treatment before respiration measurements were made.

In all cases a 15 minute equilibration period was allowed before readings of oxygen consumption were made at 15-minute intervals for 1 to 2 hours. During this period, oxygen consumption was approximately linear and the individual readings were averaged.

The effect of temperature on respiration rates of *P. f. butleri* (female) and *E. leachii* (both sexes) is shown in Fig. 1. In both species, the respiration rate of the males was slightly higher than that of the females although there were too few males of *P. f. butleri* for a statistically valid comparison to be made. For *P. f. butleri* females a mortality of about 40 per cent was noted at 35° C. *E. leachii* was not harmed by this temperature. If the data from 10° to 35° C are averaged, mean  $Q_{10}$  values were

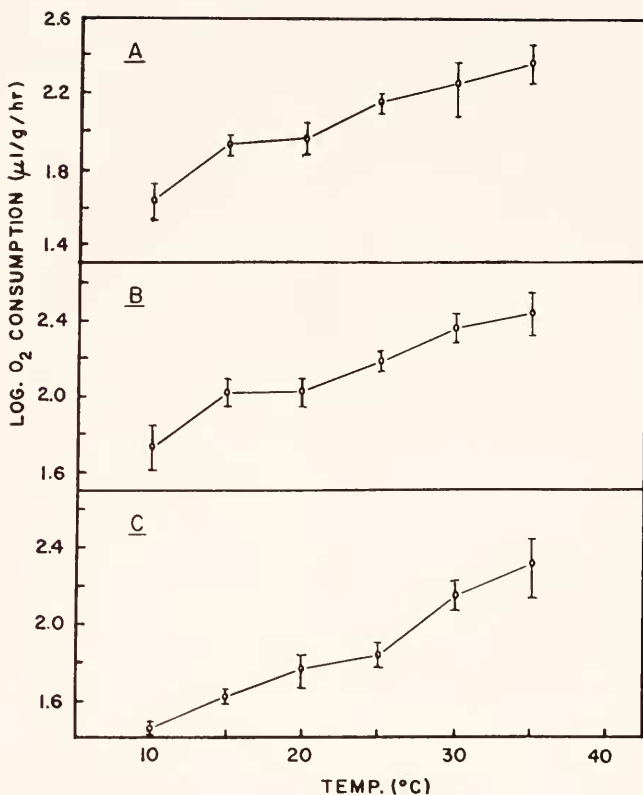


FIG. 1. Relationship of oxygen consumption to temperature in two species of millipedes. Bars represent 95% confidence levels. A. *E. leachii* (♀); B. *E. leachii* (♂); C. *P. f. butleri* (♀).

2.32 for *P. f. butleri* (female), 2.02 for *E. leachii* (male) and 1.97 for *E. leachii* (female). These values are in good agreement with the Van't Hoff relationship ( $Q_{10} = 2$ ). However, it is clear from Fig. 1 that the rise in oxygen consumption with temperature is not constant for either species. A distinct plateau occurs between 20° and 25° with *P. f. butleri* and between 15° and 20° for both sexes of *E. leachii*.

Such plateaus, often around 15°, have been found for various insects (Keister and Buck, 1964) and may sometimes be related to rearing temperature. Cloudsley-Thompson (1968) noted that several species of millipedes are observed to have a kinetic "preference" for temperatures around 15° C. However, the physiological basis and importance of such plateaus is too complex to be inferred from this brief study.

TABLE 1. Respiration rates at 25° C

	No. of Animals	Mean Wt (g)	Oxygen Consumed <sup>1</sup>
<i>P. f. butleri</i> (♀)	20	0.494	74 ± 9
<i>P. f. butleri</i> (♂)	—	—	102
<i>E. leachii</i> (♀)	18	0.188	148 ± 17
<i>E. leachii</i> (♂)	17	0.169	155 ± 18
<i>B. germanica</i> (♂)	20	0.056	702 ± 52
<i>B. discoidalis</i> (♂)	13	2.250	269 ± 15

<sup>1</sup>  $\mu\text{l O}_2$  consumed/hr/g body wt  $\pm 95\%$  confidence limits.

Comparative respiration rates of the four species at 25° C are presented in Table 1. The rates for both species of resistant millipedes are clearly well below those of the two susceptible insects, although it is worth noting that the data for *P. f. butleri* were taken when they had been maintained in the laboratory for about two months. Specimens freshly collected had respiration rates 50 to 100 per cent higher than those given here. The relatively low oxygen consumption of the millipedes may indicate that their resistance to cyanide is a further example of the excess terminal oxidase mechanism.

The oxygen consumption values for the cockroaches fall within the range quoted by Keister and Buck (1964) for non-flying insects at or around 25° C. Despite the relatively low respiration rates obtained with the millipedes, their oxygen consumption is still considerably greater than that reported for some other millipedes, e.g., 3 to 5  $\mu\text{l O}_2$  per hour per g body weight for both *Arthrosphera dalyi* (Paulpandian, 1966) and *Spirostreptus asthenes* (Dwarakanath and Job, 1965). The millipede rates are also much larger than oxygen consumption values reported for cyanide-resistant forms such as diapausing lepidopterous pupae, e.g., *Platysamia cecropia*, 16 and *Antherca pernyi*, 14  $\mu\text{l O}_2$  per hour per g body weight (Keister and Buck, 1964), but much lower than that for resistant pupal male scale insects (1,800  $\mu\text{l O}_2$  per hour per g body weight; Yust and Sheldon, 1952). Thus it may be concluded that although there is frequently a qualitative relationship between the level of respiration and ability to tolerate cyanide in invertebrates, the present work suggests that this relationship is by no means absolute. Probably this fact is an indication that different mechanisms of resistance are operating in different organisms, each having its own basal respiration rate.

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